

October 11, 1954

Dear Francis:

I think it is obvious that you are concerned (though you may rightfully feel you ought not to be) about the reaction of the mob to the Moewus-Ryan Axis. In all seriousness, though, I may have given too grave an impression in my last letter and my "solicitude" might be both unwarranted and improper. I was reporting only the worst that I had heard, not my opinions, and I am not sure how that I did the best amicable service. At least I hope you will never retaliate by repeating the worst gossip you ever hear about me. But I'm blundering again in making so much of it; I protest too much.

If anyone is to make a judgment, it should be just as you say, from an honest, and sympathetic attempt to repeat M's experiments under his own conditions. We would all like that; it might have been best if he and you could have gone into seclusion for a year or two for it. The MBL experience did work out poorly, but now I am sure you would rather be left alone to work out the matter for yourself in your own good time. What was not clear, and what I was happy to learn more explicitly, was whether you intended to let the matter hang without going into it yourself. I am glad to learn that you will; it would have been quite defensible otherwise, too, but some of the people who might have "followed M to Columbia" were confused as to your own interest in *Chlamydomonas*. I hope I can follow my own good advice and not bother you on this matter until you are ready to talk.

The Cavalli's have been great fun. Inter alii, we are doing another sort of indirect selection experiment that may interest you: 1) showing the heritability component of variance in the Luria-Delbruck analysis by comparing sets of cultures from inocula of different sizes from initial cultures whose samples contain low and high numbers of mutants, respectively, [this worked beautifully]; and 2) Executing indirect selection without replica plating as follows: if you start out with a few, m , mutants and many, n , cells, the ratio of $m:n$ can be enriched by taking samples of size x . On the average, each sample will have mx and nx respectively, of course, but if $mx < 1$, each sample individually will have either 0 or 1. The latter culture is identified later by assaying samples, and this culture will, now have $1/n$ rather than m/n rather than $m:n$, i.e., will be enriched by n/nx rather than m/nx rather than $m:n$, i.e., will be enriched by $1/nx$ rather than m/nx , i.e., by the inverse of the fraction of negative cultures. To put it differently, one is diluting both mutants and wild types in each of mx cultures but can find the specific culture that has received the mutant cell. The enrichment (for S^r) is working fairly smoothly, but slower than predicted, doubtless owing to adverse selection during growth. This will have to be checked explicitly.

I have also been playing (scarcely more) with a culture of *Aerogenes* that Hinshelwood sent me on request, as I wanted to try a "sympathetic confirmation" of his work on D-arabinose adaptation (Baskett & H. 1951). To read the paper, he might have had a long-term adaptation along the lines of

Saccharomyces/galactose (Winge & Spiegelman) possibly followed or not by a subsequent selection for an adaptively fixed mutant. Figure 2 in that experiment was the most interesting, but I have not so far been able to confirm it, primarily because a single cycle of growth in minimal-arabinose gives only a very slowly growing "first step" mutant. I do not find any (or hardly any) mutants on agar platings, much less the regular development of adapted clones from every colony. It looks very much as if there are several distinct stages of adaptation, presumably a multi-step, polygenic affair but very much as is the case for another cause celebre (which I am pledged not to mention) one may have to start from scratch even to find out the elementary facts. I doubt that I'll go into it, ~~unless~~ unless something comes up fairly soon that is a reasonable lead to a critical experiment.

Mostly, I spend my days (and some nights) over the micromanipulator, now trying to follow some pedigrees on the exconjugants of the coli matings. I haven't analysed all the results; (it's taking me a week to interpret one experiment, since even 5 or 6 or 7 generations is plenty of isolates, even if the pedigree is not complete in every line) but in a couple of cases at least, I have been surprised to find evidence for somewhat persistent (i.e., more than 6 or 8 generations but not indefinitely) heterokaryotic segregation. Usually it does not last so long. The diagnosis is based on the recurrence of a particular, rare recombinant type at widely scattered points of a pedigree, while other cells threw off some ~~mixtures~~ mixtures of the recombinant and parental or other recombinant. If the recombinant type were not rare, one could not distinguish heterokaryotic from heterozygotic segregation. It's too bad one can't do the same sort of thing not only in the statistical sense, as you have been doing, but by pedigree analysis of a mutation: perhaps this is after all what Kaplan had been running into. I have to say also that we had no evidence of this sort of thing in the pedigrees from persistent diploids, so my conclusions may be premature. I had thought a priori that fission in bacteria was probably reductional and that two or three fissions would be enough to ensure homokaryosis after mutation or segregation from a heterozygote.

Yours as ever,

Joshua.